

What is claimed is:

1. An isolated strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) and deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15<sup>th</sup> December 1997.
2. An isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.
3. The isolated nucleic acid of claim 2, wherein the polypeptide is being encoded by nucleotides 155 through 835 of the nucleic acid sequence designated SEQ. I.D. No. 1.
4. The isolated nucleic acid of claim 3, comprising nucleotides "AGA" in positions 587-589.
5. The isolated nucleic acid of claim 2, wherein the nucleic acid is DNA.
6. The isolated nucleic acid of claim 2, wherein the nucleic acid is RNA.
7. The isolated nucleic acid of claim 5, wherein the nucleic acid is cDNA.
8. The isolated nucleic acid of claim 5, wherein the nucleic acid is genomic DNA.
9. The isolated nucleic acid of claim 2, wherein the polypeptide

AMENDED SHEET

has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.

10. An isolated nucleic acid which encodes a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
11. An isolated nucleic acid which encodes a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.
12. A vector comprising an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine and operatively linked to a promoter of RNA transcription.
13. A vector comprising an isolated nucleic acid encoding a peptide, wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
14. The vector of claim 12 or 13, wherein the vector comprises viral DNA.
15. A host vector system for the production of a polypeptide which comprises the vector of claim 12 in a suitable host.
16. A host vector system for the production of a peptide which comprises the vector of claim 13 in a suitable host.

17. A method of producing a polypeptide which comprises growing the host vector system of claim 15 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.
18. A method of producing a peptide which comprises growing the host vector system of claim 16 under suitable conditions permitting production of the polypeptide and recovering the polypeptide so produced.
19. A method of obtaining a polypeptide in purified form which comprises:
  - (a) introducing the vector of claim 12 into a suitable host cell;
  - (b) culturing the resulting host cell so as to produce the polypeptide;
  - (c) recovering the polypeptide produced in step (b); and
  - (d) purifying the polypeptide so recovered.
20. A method of obtaining a polypeptide in purified form which comprises:
  - (a) introducing the vector of claim 13 into a suitable host cell;
  - (b) culturing the resulting host cell so as to produce the polypeptide;
  - (c) recovering the polypeptide produced in step (b); and
  - (d) purifying the polypeptide so recovered.
21. A purified polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15<sup>th</sup> December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.

AMENDED SHEET

22. A purified polypeptide obtained from a method which comprises:
- (a) introducing a vector comprising an isolated nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15<sup>th</sup> December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine and operatively linked to a promoter of RNA transcription into a suitable host cell;
  - (b) culturing the resulting host cell so as to produce the polypeptide;
  - (c) recovering the polypeptide produced in step (b) ; and
  - (d) purifying the polypeptide so recovered.
23. A purified peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.
24. A purified peptide obtained from a method which comprises:
- (a) introducing a vector comprising an isolated nuclei acid encoding a peptide which comprises at least a portion of a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15<sup>th</sup> December 1997 wherein the peptide is encoded by a nucleic acid molecule comprising nucleotides 527 through 595 of SEQ. I.D. No. 1 into a suitable host cell;
  - (b) culturing the resulting host cell so as to produce the polypeptide;
  - (c) recovering the polypeptide produced in step (b); and

- (d) purifying the polypeptide so recovered.
25. An oligonucleotide of at least 15 nucleotides capable of specifically hybridizing with a unique sequence of nucleotides within a nucleic acid which encodes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine, without hybridizing to any sequence of nucleotides within a nucleic acid which encodes the major surface antigen of a wildtype hepatitis B virus.
26. The oligonucleotide of claim 25 comprising nucleotides 527 through 595 of SEQ. I.D. No. 1.
27. A method of obtaining antibodies to a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and not to the major surface antigen of a wildtype hepatitis B virus, comprising:
- (a) obtaining the polypeptide in a purified form;
  - (b) immunizing an organism capable of producing antibodies against the purified polypeptide;
  - (c) collecting the produced antibodies;
  - (d) combining the produced antibodies and the purified polypeptide under conditions to form a complex; and
  - (e) determining which produced antibodies form a complex with the purified polypeptide so as to obtain antibodies to the polypeptide.

28. The method of claim 27, wherein the polypeptide is being encoded by nucleotides 155 through 835 of the nucleic acid sequence designated SEQ. I.D. No. 1.
29. The method of claim 27, wherein the polypeptide has an amino acid sequence substantially the same as amino acid residues 174 through 400 of the amino acid sequence designated SEQ. I.D. No. 3.
30. The method of claim 27, wherein the organism comprises a rabbit or a mouse.
31. A method of obtaining antibodies to a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3, comprising:
  - (a) obtaining the peptide in a purified form;
  - (b) immunizing an organism capable of producing antibodies against the purified peptide;
  - (c) collecting the produced antibodies;
  - (d) combining the produced antibodies and the purified peptide under conditions to form a complex; and
  - (e) determining which produced antibodies form a complex with the purified peptide so as to obtain antibodies to the peptide.
32. The method of claim 31, wherein the organism comprises a rabbit or a mouse.
33. The antibodies obtained in claim 27 or 31.
34. Monoclonal antibodies of the antibodies of claim 33.
35. Antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus,

such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and incapable of detecting the major surface antigen of a wildtype hepatitis B virus.

36. Antibodies capable of detecting a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3.
37. Use of a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such determination comprises
  - (a) obtaining an appropriate nucleic acid sample from the subject; and
  - (b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine.
38. The use of claim 37, wherein the nucleic acid sample in step

(a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

- (i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;
- (ii) isolating the complex so formed; and
- (iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

39. The use of claim 37, wherein the nucleic acid sample in step (a) comprises mRNA corresponding to the transcript of DNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

- (i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and
- (ii) comparing the amino acid sequence of step (i) with the amino acid sequence encoded by the isolated nucleic acid of claim 9 so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.



40. The use of claim 37, wherein the determining of step (b) comprises:
- (i) amplifying the nucleic acid present in the sample of step (a); and
  - (ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.
41. Use of antibodies capable of detecting a polypeptide which is a mutant major surface antigen of a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) for determining whether a subject is infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein such determination comprises:
- (a) obtaining an appropriate sample from the subject; and
  - (b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 or 36 so as to determine whether a subject is infected.
42. The use of claim 37, 38 or 41, wherein the isolated nucleic acid, oligonucleotide, or antibody is labeled with a detectable marker.
43. The use the claim 42, wherein the detectable marker is a radioactive isotope, a fluorophor, or an enzyme.

68. A method of obtaining a composition which comprises a identifying a chemical compound by the method of claim 66 and admixing the compound with a pharmaceutically effective carrier.
69. Use of the composition of claim 47 or 48 as a medicament for treating hepatocellular carcinoma.
70. Use of the composition of claim 67 as a medicament for treating hepatocellular carcinoma.
71. Use of the composition of claim 47 or 48 as a medicament for preventing hepatocellular carcinoma.
72. Use of the composition of claim 67 as a medicament for preventing hepatocellular carcinoma.
73. A hepatitis vaccine, comprising a mutant form of the surface antigen of hepatitis B virus deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15<sup>th</sup> December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of the major surface antigen of hepatitis B in that the amino acid at position number 145 of such polypeptide is an arginine rather than a glycine.
74. The vaccine of claim 73, further comprising an adjuvant.

RECEIVED 1997

44. The use of claim 37, wherein the sample comprises blood, tissue, or sera.
45. A method for identifying a chemical compound for use in the manufacture of a medicament capable of treating infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein the method for identifying the chemical compound comprises:
- (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;
  - (b) detecting specific binding of the chemical compound to the polypeptide; and
  - (c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of treating infection by the viral strain.
46. A method for identifying a chemical compound for use in the manufacture of a medicament capable of preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine), wherein the method for identifying the chemical compound comprises:
- (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen

- of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;
- (b) detecting specific binding of the chemical compound to the polypeptide; and
  - (c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of preventing infection by the viral strain.
47. A composition comprising a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus deposited under Accession Nos. P97121501, P97121502 and P97121503 with the European Collection of Cell Culture on 15<sup>th</sup> December 1997, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, the amounts of such polypeptide being effective to stimulate or enhance antibody production in a subject, and a pharmaceutically acceptable carrier.
48. A composition comprising a peptide, wherein the peptide has an amino acid sequence comprising amino acid residues 298 through 320 of the amino acid sequence designated SEQ. I.D. No. 3., the amounts of such peptide being effective to stimulate or enhance antibody production in a subject, and a pharmaceutically acceptable carrier.
49. A method of obtaining a composition which comprises a identifying a chemical compound by the method of claim 45 and admixing the compound with a pharmaceutically effective carrier.

50. A method of obtaining a composition which comprises a identifying a chemical compound by the method of claim 46 and admixing the compound with a pharmaceutically effective carrier.
51. Use of the composition of claim 47 or 48 for treating a subject infected with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine).
52. Use of the composition of claim 49 for treating a subject infected with a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine).
53. Use of the composition of claim 47 or 48 for preventing infection by a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) in a subject.
54. Use of the composition of claim 50 for preventing infection with a strain of Hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) in a subject.
55. A method of screening bodily fluids from a subject for a strain of hepatitis B virus designated Human Hepatitis B Virus Surface Antigen-'S'-145 Singapore Strain (Glycine to Arginine) which comprises:
  - (a) obtaining an appropriate sample of bodily fluid from the subject;
  - (b) determining the presence of a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major

surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, in the sample of step (a) so as to screen the sample for the strain.

56. The method of claim 55, wherein the bodily fluid comprises blood, sera, or a nucleic acid sample of blood or sera.
57. Use of an antibody that recognizes a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determination comprises:
  - (a) obtaining an appropriate nucleic acid sample from the subject; and
  - (b) determining whether the nucleic acid sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 35 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.
58. The method of claim 57, wherein the nucleic acid sample in step (a) comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a

glycine, and wherein the determining of step (b) comprises:

- (i) contacting the mRNA with the oligonucleotide of claim 25 under conditions permitting binding of the mRNA to the oligonucleotide so as to form a complex;
- (ii) isolating the complex so formed; and
- (iii) identifying the mRNA in the isolated complex so as to thereby determine whether the mRNA is, or is derived from, a nucleic acid which encodes the polypeptide.

59. The method of claim 57, wherein the nucleic acid sample in step (a) comprises mRNA encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, and wherein the determining of step (b) comprises:

- (i) translating the mRNA under suitable conditions to obtain an amino acid sequence; and
- (ii) comparing the amino acid sequence of step (i) with the amino acid sequence encoded by the isolated nucleic acid of claim 9 so as to determine whether the nucleic acid sample is, or is derived from, a nucleic acid which encodes the polypeptide.

60. The method of claim 57, wherein the determining of step (b) comprises:

- (i) amplifying the nucleic acid present in the sample of step (a); and
- (ii) detecting the presence of polypeptide in the resulting amplified nucleic acid.

61. Use of an antibody that recognizes a polypeptide which is a

mutant major surface antigen of a strain of hepatitis B virus for determining whether the subject has a predisposition for hepatocellular carcinoma, wherein such determination comprises:

- (a) obtaining an appropriate sample from the subject; and
- (b) determining whether the sample from step (a) is, or is derived from, a nucleic acid encoding a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, by contacting the sample under appropriate conditions to bind to the antibodies of claim 36 so as to determine whether the subject has a predisposition for hepatocellular carcinoma.

- 62. The method of claim 58, 59 or 60, wherein the oligonucleotide or antibody is labeled with a detectable marker.
- 63. The method of claim 62, wherein the detectable marker is a radioactive isotope, a fluorophor or an enzyme.
- 64. The method of claim 57, wherein the sample comprises blood, tissue or sera.
- 65. A method for identifying a chemical compound for use in the manufacture of a medicament capable of treating hepatocellular carcinoma wherein the method for identifying the chemical compound comprises:
  - (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen



of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;

- (b) detecting specific binding of the chemical compound to the polypeptide; and
- (c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of treating hepatocellular carcinoma.

66. A method for identifying a chemical compound for use in the manufacture of a medicament capable of preventing hepatocellular carcinoma, wherein the method for identifying the chemical compound comprises:

- (a) contacting a polypeptide which is a mutant major surface antigen of a strain of hepatitis B virus, such polypeptide having an amino acid sequence which differs from the amino acid sequence of a major surface antigen of a wildtype hepatitis B virus in that the amino acid at position number 145 of such polypeptide is an arginine, rather than a glycine, with the chemical compound under conditions permitting binding between the polypeptide and the chemical compound;
- (b) detecting specific binding of the chemical compound to the polypeptide; and
- (c) determining whether the chemical compound binds to the polypeptide so as to identify a chemical compound which is capable of preventing hepatocellular carcinoma.

67. A method of obtaining a composition which comprises a identifying a chemical compound by the method of claim 65 and admixing the compound with a pharmaceutically effective carrier.